Trials and tribulations of MLF: can timing of inoculation and MLF nutrients help?

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Co-inoculation of malolactic starter cultures and the use of specific malolactic nutrients are two approaches aimed at improving the efficiency of malolactic fermentation. This report summarises the main findings of several winery-scale studies carried out over two vintages investigating the effects of these techniques on MLF efficiency and wine chemical and sensory properties.

INTRODUCTION

Malolactic fermentation (MLF) is an important secondary fermentation conducted in most red wines and some white and sparkling base wines. Characterised by the conversion of malic acid to lactic acid and carbon dioxide, MLF is carried out by certain lactic acid bacteria, predominantly *Oenococcus oeni.* While MLF is a common process in winemaking, it can be unpredictable, with delayed or slow MLFs causing problems for winemakers. Ongoing research aimed at gaining greater control over MLF has led to important developments including direct inoculation malolactic starter cultures (Nielsen *et al.* 1996). Nevertheless, MLF failure or delay may still occur, particularly in challenging wine conditions such as those with a high alcohol content, low pH and moderate to high concentrations of SO_2.

One strategy aimed at improving MLF efficiency is co-inoculation, where a bacterial starter culture is added 24 to 48 hours after the ferment is inoculated with yeast. By this approach, MLF can initiate simultaneously with alcoholic fermentation (AF) under the favourable conditions of lower alcohol content and greater availability of nutrients. Co-inoculation has also been shown to enable MLF induction in difficult wine conditions including low pH white wine (Jussier *et al.* 2006, Knoll *et al.* 2012, Guzzon *et al.* 2016) and high alcohol red wine (Zapparoli *et al.* 2009). In some circumstances, however, it may also be associated with risks including minor increases in acetic acid and negative interactions with fermentation yeasts (see reviews by Sumby *et al.* 2014, Bartowsky *et al.* 2015). Nevertheless, with use of compatible yeast and bacteria pairs and careful fermentation control, co-inoculation is generally considered a practical option for improving fermentation efficiencies.

Another strategy aimed at enhancing malolactic starter culture performance is the use of specific MLF nutrients. Such preparations are advocated to support the complex nutritional requirements of malolactic bacteria, and have been shown to benefit MLF efficiency in certain wines (Deleris-Bou and Krieger-Weber 2015). However, information on the application of commercial malolactic nutrients is limited and further research is required to assess their performance.

This report provides an overview of several winery-scale trials conducted by the AWRI investigating the effects of co-inoculation and malolactic nutrients on MLF performance and wine chemical and sensory properties. These studies were undertaken in Shiraz and Chardonnay sparkling base wines in collaboration with two commercial wineries over the 2016 and 2017 vintages.

INOCULATION TIMING: CO-INOCULATION VS SEQUENTIAL INOCULATION

Shiraz wine

Timing of inoculation in Shiraz from Langhorne Creek was investigated in the 2016 and 2017 vintages using malolactic starter cultures prepared from AWRI *Oenococcus oeni* strains. Shiraz grapes were crushed into three stainless steel open fermenters (10 tonne) for co-inoculation, sequential inoculation or natural MLF, and the must was then transferred into 225L oak barrels (duplicates) and maintained at approximately 20°C for the duration of MLF. In these trials, co-inoculation was found to be effective in reducing overall fermentation time, particularly in the 2016 Shiraz where co-inoculation with strain AWRI B447 required only 10 days to complete MLF, compared with sequential inoculation (more than 100 days) and natural MLF (80 days) (Figure 1). In 2017, MLF was also completed more rapidly via co-inoculation than sequential inoculation, with both strains AWRI B706 (33 days vs 40 days) and AWRI B447 (36 days vs 54 days), and natural MLF requiring more than 80 days (Figure 2). Differences in time to complete MLF between the two vintages could reflect differences in must/wine composition and in starter culture acclimatisation protocols.

The chemical composition of the Shiraz wines following MLF (Tables 1

AT A GLANCE

- Winery-scale experiments in Shiraz and sparkling base wines examined factors influencing malolactic fermentation (MLF) efficiency and flavour outcomes.
- Timing of inoculation (co-inoculation, sequential inoculation and natural MLF) and nutrient additions were investigated.
- In some cases, co-inoculation improved MLF efficiency and led to the shortest times to complete MLF. Choice of bacterial strain was also important in the efficiency of co-inoculations.
- Addition of malolactic nutrients did not improve MLF efficiency in the wines studied.
- Inoculation timing and addition of nutrients both had sensory effects on the final wines.

and 2) indicates that inoculation timing had minimal effect on major wine compositional parameters. In 2016, the VA content of the co-inoculation treatment (0.41g/L) was marginally higher than that of the sequential inoculation treatment (0.36g/L) and the natural MLF treatment (0.29g/L), while there were minimal differences in VA in the 2017 trial (range 0.34-0.41g/L). Some variation in diacetyl content also occurred in the 2016 trial, where wines from co-inoculation (8.4mg/L) were higher than sequential inoculation (1.8mg/L) or natural MLF (4.0mg/L) treatments. Although the sensory threshold of diacetyl in red (Cabernet Sauvignon) wine is 2.8mg/L (Martineau *et al.* 1995), there were no sensory differences relating to 'butteriness' observed in these wines.

Chardonnay sparkling base wine

The effect of inoculation timing was also investigated in a 2017 Tasmanian Chardonnay sparkling wine base. Following yeast inoculation, Chardonnay must was transferred to 230L stainless steel or oak barrels for MLF treatments with four different strains of *O. oeni*. The lengthy duration of MLF observed with this wine reflects its harsh chemical conditions (pH3.03, 24mg/L total SO_2). Significantly, there were no consistent trends of the effects of inoculation timing on fermentation efficiency, with much variation associated with choice of bacterial strain (Figure 3, see page 40). In contrast, Knoll *et al.* (2012) found that total fermentation time (AF + MLF) was shorter with simultaneous inoculations than sequential inoculations in Riesling must/wine with low pH (2.9-3.1). These authors observed, however, that the length of MLF with simultaneous and sequential inoculation was also influenced by the choice of bacterial strain. Generally, the effects of inoculation timing on wine chemical composition including VA and diacetyl content in the current study were minimal (Table 3, see page 40). However, compared with the other treatments, wine treated by co-inoculation with the commercial *O. oeni* strain (B) was observed to have a relatively higher total acidity (9.2g/L) and VA (0.7g/L), and slightly lower pH (3.10) (Table 3).

Sensory assessment of both the Shiraz and sparkling base wines did not reveal any clear influence of inoculation

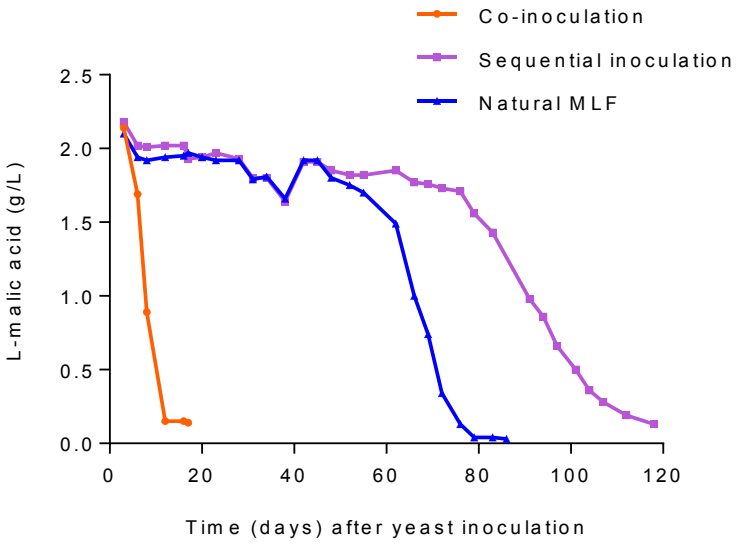

Figure 1. Influence of timing of bacterial inoculation with *Oenococcus oeni* AWRI B706 on duration of MLF in 2016 Langhorne Creek Shiraz.

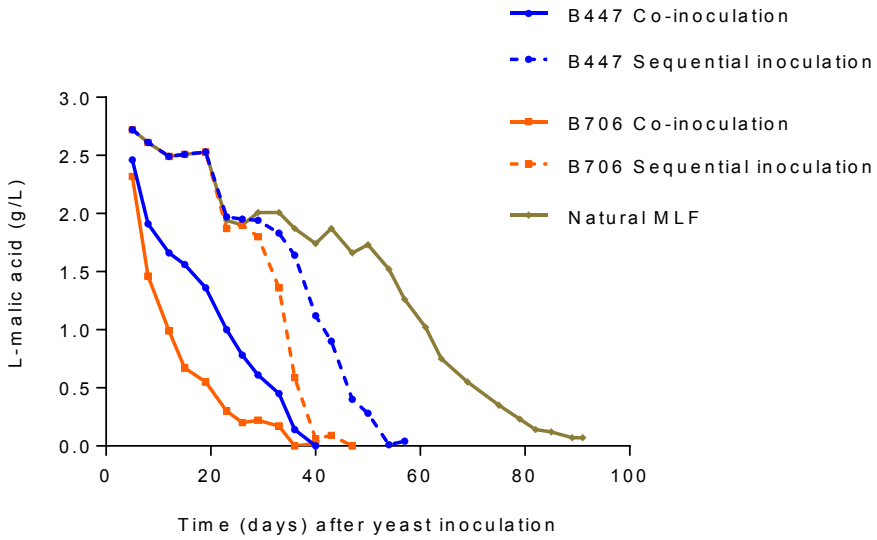

Figure 2. Influence of timing of bacterial inoculation with *Oenococcus oeni* strains AWRI B706 and AWRI B447 on MLF duration in 2017 Langhorne Creek Shiraz.

Table 1. Effect of timing of bacterial inoculation on chemical composition of 2016 Langhorne Creek Shiraz following MLF with *Oenococcus oeni* AWRI B447 and natural MLF.

Treatment	Alcohol (% v/v)	pH	TA (g/L)	VA (g/L)	Diacetyl (mg/L)
Simultaneous	13.1	3.56	6.8	0.41	8.4
Sequential	13.1	3.59	6.9	0.36	1.8
Natural	13.3	3.64	6.5	0.29	4.0

Table 2. Effect of timing of bacterial inoculation on chemical composition of 2017 Langhorne Creek Shiraz following MLF with *Oenococcus oeni* AWRI B447, AWRI B706 and natural MLF.

Bacteria strain/ treatment	Alcohol (%v/v)	pH	TA (g/L)	VA (g/L)	Glucose + Fructose (g/L)	Diacetyl (mg/L)
B447 (SIM)	14.7	3.70	6.0	0.34	0.2	6.1
B447 (SEQ)	15.4	3.72	6.1	0.41	0.3	7.3
B706 (SIM)	15.2	3.67	6.2	0.43	0.3	15.0
B706 (SEQ)	15.4	3.72	6.1	0.42	0.3	10.0
NATURAL	15.3	3.67	6.4	0.40	0.3	11.0

Table 3. Influence of bacterial inoculation timing and bacterial strain on chemical composition of 2017 Chardonnay sparkling wine base.

	Wine (post -alcoholic fermentation)	Wine (post-MLF)							
		AWRI B706		AWRI B447		AWRI B1062		Commercial *O. oeni* (B)	
		Co-inoculation	Sequential	Co-inoculation	Sequential	Co-inoculation	Sequential	Co-inoculation	Sequential
Alcohol (% v/v)	11.5	-	-	-	-	-	-	-	-
pH	3.03	3.17	3.18	3.18	3.17	3.17	3.18	3.10	3.18
TA (g/L)	11.8	8.4	8.5	8.4	8.5	8.5	8.3	9.2	8.4
Total SO2 (mg/L)	24	-	-	-	-	-	-	-	-
G+F (g/L)	0.6	0.7	0.9	0.7	0.8	0.7	0.9	0.7	0.7
VA (g/L)	0.21	0.3	0.31	0.29	0.28	0.34	0.35	0.5	0.36
Diacetyl (mg/L)	-	0.2	0.3	0.2	0.1	0.1	0.2	0.2	0.3

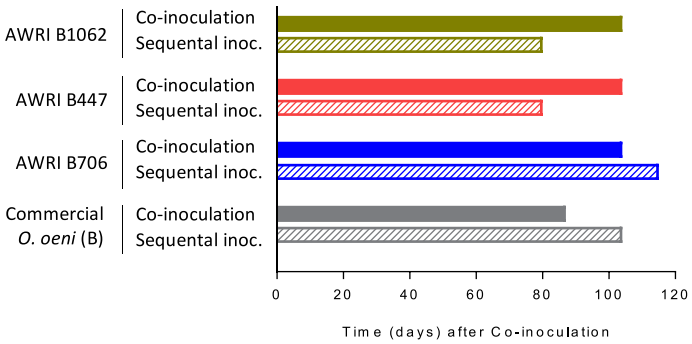

Figure 3. Influence of timing of bacterial inoculation on MLF duration for different *Oenococcus oeni* strains in 2017 Chardonnay sparkling wine base.

timing on wine sensory properties, which may have been related to some artefacts associated with post-fermentation handling of the wines. However, compared with simultaneous inoculation, 'overall fruit flavour' in the 2016 Langhorne Creek Shiraz was significantly higher in wines with sequential inoculation. Other reports have generally shown, however, that 'fruity' sensory properties and concentrations of flavour compounds contributing to fruitiness were favoured by co-inoculation (see reviews by Bartowsky *et al.* 2015, Sumby *et al.* 2014). Nevertheless, the results from the 2016 Langhorne Creek Shiraz trial support the work of (Antalick *et al.* 2013) who found that under winery conditions, co-inoculation did not necessarily favour 'fruity' expression or lower diacetyl content in Merlot wine.

EFFECTS OF NUTRIENT SUPPLEMENTATION

The effect of two commercial malolactic nutrient preparations on MLF performance was investigated in two different 2016 wines, a Shiraz and a Chardonnay sparkling base wine, both with challenging conditions for MLF. In the first study, four different treatment combinations, with/without commercial malolactic nutrient (N1) supplementation and natural/inoculated MLF (commercial *O. oeni* A), were examined in a Barossa Valley Shiraz wine (15.1% v/v alcohol, pH 3.55). Treatments (sequential inoculation) were undertaken in oak barrels (300L), with four replicate barrels per treatment. From the results (Figure 4), it is clear that the addition of malolactic nutrient did not influence the time required to complete MLF, either for wines inoculated with malolactic starter culture (both 44 days) or wines that underwent natural MLF (62 and 65 days).

In the second study, the effect of a commercial malolactic nutrient (N2) on MLF efficiency was investigated following alcoholic fermentation in a Tasmanian Chardonnay sparkling base wine (pH3.17, 11.6% v/v alcohol, 38mg/L total SO_2). The trial wine was transferred into four 230L oak barrels: two barrels were supplemented with nutrient and two others served as non-supplemented controls. Malolactic fermentation was initiated by inoculation of a bacterial starter culture (AWRI B706) and the wines were stored at approximately 20°C over the course of the MLF. The results showed that nutrient supplementation did not have any clear effect on MLF progress (Figure 5), with the time required for MLF in the non-supplemented control (48 days) slightly shorter than that of the nutrient-supplemented treatment (59 days).

The lack of any stimulatory effect of nutrient addition on MLF in these studies suggests that their nutritional composition was not limiting, and was sufficient for the survival and growth of the malolactic bacteria populations. The nutrient treatments did, however, appear to have some sensory effect. In the Shiraz wine, treatments with nutrient addition had a higher response for 'overall fruit', 'red and dark fruit' and 'buttery' aroma and flavour. In contrast, nutrient addition to the Chardonnay sparkling base wine resulted in a significantly ($p< 0.05$) lower 'buttery' aroma and flavour, and lower 'overall fruit' aroma and flavour. There was no apparent correlation between changes in 'buttery' aroma and flavour and diacetyl content in either wine (data not shown), suggesting that the concentrations of other flavour compounds contributing to this attribute may have been modulated by nutrient treatment.

CONCLUSIONS

The case studies presented here show that under certain wine conditions, co-inoculation can improve MLF efficiency and overall fermentation time in winery-scale fermentations. However, the efficacy of co-inoculation can be further influenced by other factors including the choice of bacterial strain, and may be limited in stressful wine conditions. Further, the use of malolactic nutrients did not improve MLF efficiency in the wines studied. Wine sensory properties may be influenced by inoculation timing, bacterial strain and use of malolactic nutrient. Further winery-scale studies are required to gain clearer insight into the effects of inoculation timing and use of MLF nutrients on fermentation efficiencies and wine chemical and sensory properties.

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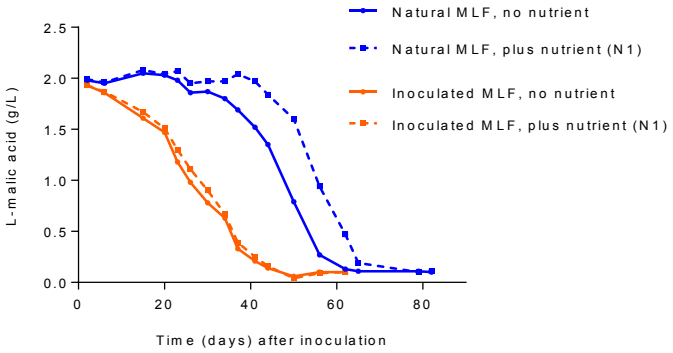

Figure 4. Effect of malolactic nutrient N1 on MLF conducted by *Oenococcus oeni* (A) or natural MLF in 2016 Barossa Valley Shiraz. Data are averages of four replicate barrels.

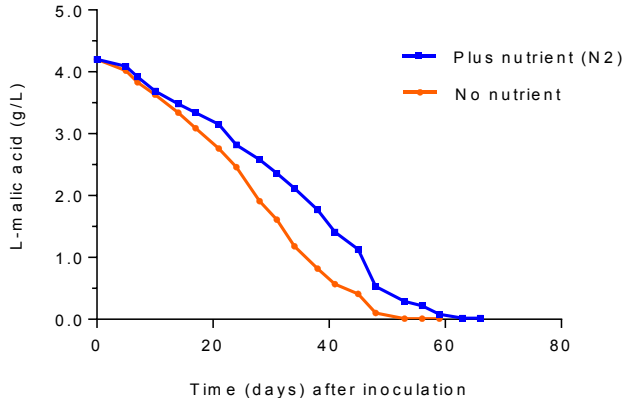

Figure 5. Effect of malolactic nutrient (N2) on MLF progress by *Oenococcus oeni* AWRI B706 in 2016 Chardonnay sparkling base wine. Data are averages of two replicate barrels.